Downloaded from orbit.dtu.dk on: May 05, 2023

DTU Library

Effects of Modified DATEMs with Different Alkyl Chain Lengths on Improving Oxidative and Physical Stability of 70% Fish Oil-in-Water Emulsions

Yesiltas, Betül; García-Moreno, Pedro Jesús; Moltke Sørensen, Ann-Dorit ; Anankanbil, Sampson; Guo, Zheng; Jacobsen, Charlotte

Published in:
Journal of Agricultural and Food Chemistry

Link to article, DOI:
10.1021/acs.jafc.8b04091

Publication date:
2018

Document Version
Peer reviewed version

Link back to DTU Orbit

Citation (APA):
Yesiltas, B., García-Moreno, P. J., Moltke Sørensen, A-D., Anankanbil, S., Guo, Z., & Jacobsen, C. (2018). Effects of Modified DATEMs with Different Alkyl Chain Lengths on Improving Oxidative and Physical Stability of 70% Fish Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry*, *66*(47), 12512–12520. https://doi.org/10.1021/acs.jafc.8b04091

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

DTU Library
Technical Information Center of Denmark

Subscriber access provided by DTU Library

Bioactive Constituents, Metabolites, and Functions

Effects of modified DATEMs with different alkyl chain lengths on improving oxidative and physical stability of 70% fish oil-in-water emulsions

Betül Yesiltas, Pedro J. Garcia-Moreno, Ann-Dorit Moltke Sørensen, Sampson Anankanbil, Guo Zheng, and Charlotte Jacobsen

J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.8b04091 • Publication Date (Web): 06 Nov 2018

Downloaded from http://pubs.acs.org on November 7, 2018

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

Effects of modified DATEMs with different alkyl chain lengths on improving oxidative and physical stability of 70% fish oil-in-water emulsions

Betül Yesiltas[1], Pedro J. García-Moreno[1], Ann-Dorit M. Sørensen[1], Sampson Anankanbil[2], Zheng Guo[2], Charlotte Jacobsen[1]

[1]National Food Institute, Technical University of Denmark, Lyngby, Denmark

[2]Department of Engineering, Aarhus University, Aarhus, Denmark

*Correspondence: Charlotte Jacobsen, National Food Institute, Technical University of Denmark, Kemitorvet, Building 204, 2800 Kgs. Lyngby, Tel: +45 23 27 90 75; e-mail: chja@food.dtu.dk

Abstract

The objective of this study was to produce oxidatively and physically stable 70% fish oil-in-water emulsions by combined use of sodium caseinate (CAS), commercial diacetyl tartaric acid esters of mono- and diglycerides (DATEM), and modified DATEM. First, optimal formula was determined using DATEM and CAS. Subsequently, modified DATEMs (DATEM C12 and DATEM C14) were designed for investigating both the effects of different alkyl chain lengths and caffeic acid conjugation to the emulsifier on physical and oxidative stability of the emulsions. Emulsions produced with modified DATEMs showed better oxidative stability compared to emulsion with commercial DATEM plus equivalent amount of free caffeic acid, confirming the advantage of having antioxidant covalently attached to the emulsifier. Results indicated that DATEM_C14 replaced more CAS compared to DATEM_C12 from the interface in 70% fish oil-in-water emulsion. Emulsions produced with DATEM_C14 had significantly decreased amounts of primary and secondary oxidation products compared to emulsions with DATEM_C12.

Key words: modified emulsifiers; DATEM and sodium caseinate; lipid oxidation; oil-water interface; caffeic acid; high fat delivery emulsions

1. Introduction

There has been increasing evidences on health benefits of long chain (LC) omega-3 (n-3) polyunsaturated fatty acids (PUFAs), such as eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA). These health benefits are mainly reducing the risk of cardiovascular diseases,[1,2] maintaining normal blood pressure and triglyceride levels in the blood[3], improving brain development in the infant[4], and supporting mental health[5] as well as immune system.[6] However, consumption of these bioactive compounds has been reported to be below daily recommended levels.[3] Therefore, there is high interest from food researchers and industry to enrich food products with LC n-3 PUFAs. Nevertheless, LC n-3 PUFAs are highly prone to oxidation and there are many factors affecting oxidation in complex food systems such as mayonnaise, milk, cream cheese and dressings, which are basically oil-in-water emulsions. This has led researchers to study lipid oxidation in fish-oil-in water emulsions, which have been used as a delivery system for LC n-3 PUFAs.[7-9] The most important factors affecting lipid oxidation are type and concentration of the ingredients (e.g., oil, emulsifiers, etc.), antioxidants existing or added in the system, oil-water interface structure and distribution of emulsifiers and antioxidants in the emulsion, surface charge of the oil droplets and other physical parameters of oil-in-water emulsions such as droplet size and viscosity. [7-9]

Different approaches have been suggested for protecting emulsified lipids from oxidation and improving the properties of the oil-water interfacial layer is one of them. Previous studies claimed that oxidation is initiated at the oil-water interface. Therefore, attention has been focused on enhancing interfacial layer capabilities such as increasing antioxidant activities at

the interface as well as using combination of emulsifiers, which are expected to provide better interfacial characteristics.[8,10] In order to enhance the physical structure of interface layer, proteins and low molecular weight emulsifiers (LMWEs) are suggested to be combined together for the emulsification of oils.[11,12] Sodium caseinate (CAS), as a milk protein and a common emulsifier in food industry, has been combined with LMWEs (e.g., phospholipids, mono- and diglycerides, etc.). This approach resulted in displacement of adsorbed proteins at the oil-water interface by the LMWEs, which also resulted in the ability of the LMWEs to help covering the interface through the small gaps between proteins.[11,13,14] This might lead to a well-covered oil-water interface by the formation of protein-phospholipid complexes, which then prevents migration of pro-oxidants through the interfacial layer from the water phase to the oil phase, where LC n-3 PUFAs are located.

In order to improve the oxidative stability of emulsions, phenolic acids have been used due to their antioxidant properties. Sørensen et al. studied the effects of adding phenolic compounds into low fat (10%) fish oil-in-water emulsions and investigated their interaction with emulsifiers as well as effects of pH and iron addition on oxidative stability of LC n-3 PUFAs.[15] Moreover, researchers also focused on adding phenolipids in the emulsion systems to obtain better oxidative stability by having antioxidant effects at the oil-water interface. Phenolipids are expected to be surface active due to their amphiphilic character and adsorb at the oil-water interface where the oxidation is claimed to be initiated by prooxidants, e.g. metal ions. The effects of having different chain lengths of phenolipids on oxidative stability have been studied in different emulsion systems.[16-22] The different efficacies of phenolipids with different chain

lengths were related to their location/distribution in the emulsion system, oil phase, oil-water interface and aqueous phase.

Caffeic acid acts as an antioxidant by scavenging free radicals and chelating metals especially iron.[23] Caffeic acid efficacy in O/W emulsions was shown to be dependent on pH, iron addition and emulsifier type.[15] Antioxidant properties and efficacy of alkyl caffeates, ferulates, and coumarates have been investigated and only caffeic acid and caffeates were able to form a complex with iron via their catechol group in the phenolic ring.[19] The same study also reported that caffeic acid and alkyl caffeates showed the highest radical scavenging activity and reducing power, which was followed by ferulic acid and alkyl ferulates, whereas coumaric acid and alkyl coumarates showed the lowest efficiency. It was also shown that the medium alkyl chain length (octyl caffeate) was found to have higher antioxidant activity than shorter (caffeic acid, methyl-, propyl caffeate) or longer (hexadecyl caffeate) alkyl chains, which was related to their presence at the interfacial region.[22] Alemán et al. also showed that caffeates with `short to medium´ chain (C4, C8 and C12) were found to be more effective antioxidants in fish oil enriched mayonnaise.[18]

Commercial DATEM is a commonly used surfactant in food industry, mainly in baking. In order to combine advantageous effects of both caffeates and DATEM (antioxidative and surface active effects, respectively), we aimed to use modified DATEM as a LMWE, which was modified with caffeic acid, and C12 or C14 alkyl chains, into high fat fish oil-in-water emulsions stabilized in combination with CAS. Chain lengths were selected according to the results from previous studies conducted with phenolipids which showed that the `short to medium´ chain length (C4-C12) performed the best[18]. Considering the larger head part of the

DATEM molecules compared to a phenolipid, we decided to study the longest `short to medium´ chain length, which is C12 and one even longer (C14) in order to adjust the hydrophilic-lipophilic balance (HLB) of the molecules. A recent study reported that DATEM C14 led to lower lipid oxidation (TBARS) when compared to DATEM C12 or C16 in 30% fish oil-in-water emulsions.[24]

The first part of this study focused on finding an optimal recipe for emulsifying high fat fish oil-in-water emulsions using CAS and commercial DATEM. For that purpose we used Box-Behnken`s design combined with Response Surface Methodologies (RSM). The effects of fish oil content, emulsifier content and ratio of CAS to DATEM on physical and oxidative parameters were evaluated. The second part of the study aimed at investigating the use of CAS in combination with different concentrations of modified DATEMs with caffeic acid and different fatty acid chain lengths (C12 and C14). The use of modified DATEM was compared to commercial DATEM with and without free caffeic acid to evaluate the effect of having: i) caffeic acid attached to the emulsifier on oxidative stability of high-fat fish oil-in-water emulsions, and ii) different chain lengths on distribution of emulsifiers in high-fat emulsion systems as well as its effect on physical and oxidative stability.

2. Materials and Methods

2.1. Materials

Cod liver oil used for the optimization experiment was provided by Maritex A/S, subsidiary of TINE, BA (Sortland, Norway), and stored at -40ºC until use. The fatty acid (%, w/w) content of the fish oil was as follows: C14:0 (3.0), C16:0 (8.9), C16:1 n-7 (8.2), C18:0 (1.9), C18:1 n-9

(16.0), C18:1 n-7 (5.2), C18:2 n-6 (1.8), C18:3 n-3 (0.8), C20:1 n-9 (11.6), C20:5 n-3 (9.3), C22:1 n-11 (6.1), and C22:6 n-3 (11.6). Cod liver oil used for modified DATEM experiment was provided by Vesteraalens A/S (Sortland, Norway) and stored at -40 ºC until use. Peroxide value was determined as 0.12±0.08 meq peroxides/kg oil. The fatty acid (%, w/w) content of the fish oil was as follows: C14:0 (0.2), C16:0 (9.4), C16:1 n-7 (8.6), C18:0 (2.0), C18:1 n-9 (16.2), C18:1 n-7 (4.6), C18:2 n-6 (1.8), C18:3 n-3 (0.1), C20:1 n-9 (12.6), C20:5 n-3 (9.1), C22:1 n-11 (5.9), and C22:6 n-3 (11.1). Alpha-, beta-, gamma-, and delta tocopherol contents were 250±2, 0±0, 118±1, 48±1 μg toc/g oil, respectively. Sodium caseinate, CAS (Miprodan 30) was kindly donated by Arla Foods Ingredients amba (Viby J, Denmark). Arla reported a protein content of 92% in sodium caseinate for Miprodan 30. DATEM (PANODAN AB 100 VEG-FS MB, PD 244-18.3 EN) was provided by Danisco (Brabrand, Denmark). Caffeic acid was purchased from Sigma Aldrich. Modified DATEMs with caffeic acid and different alkyl chains C12 or C14 were synthesized as described in a previous study.[25] All other chemicals and solvents used were of analytical grade.

2.2. Experimental designs

2.2.1. Experimental design for optimal formula determination

The optimal recipe was determined by Box-Behnken design using three different levels for three factors which were fish oil content (50, 60, and 70%), total emulsifier content (1.4, 2, and 2.8%) and the ratio between CAS and DATEM (0.4, 1.2, and 2). Experimental design and details are shown in the Supplementary material.

2.2.2. Experimental design for emulsions produced with modified DATEMs

Sample codes and sample descriptions amounts of added ingredients are listed in Table 1. All samples include 70 wt% fish oil and 2.8 wt% total emulsifier with a ratio of CAS to total DATEM of 2. Commercial DATEM was replaced by modified DATEM in a range of 0 to 60%. Emulsions presenting commercial DATEM and free caffeic acid were also added as controls.

2.3. Emulsion preparation and sampling

Prior to emulsification, emulsifiers (CAS, DATEM, and modified DATEMs) were dissolved in distilled water and stirred overnight at 4˚C. Aqueous phases were adjusted to pH 7 using 1M HCl and 2M NaOH. Emulsions were produced in 500 g batches in a Stephan Universal mixer (Stephan, UMC5, 1995, Hameln, Germany) as explained by Horn et al.[27] Sodium azide (0.05% w/v) and 100 μM Fe^{2+} were added into emulsions in order to prevent microbial activity and accelerate lipid oxidation, respectively. All emulsions were divided into 100 mL bottles in approximately 90 g and stored at room temperature for up to 12 days in the dark. Samples were collected at days 0, 2, 5, 8 and 12 for oxidative stability analysis.

2.4. Methods for characterization of emulsions

2.4.1. Creaming index

Creaming was measured on days 1, 5, 8 and 12 in the stored bottles without replicates. Creaming index was calculated using equation 1:

$$CI\ (\%) = b/a*100 \qquad \text{(Equation 1)}$$

where (a) is the height of total emulsion and (b) is the height of water phase separated at the bottom of the bottle. Creaming index was calculated as in percentage for each emulsion sample and sampling point.

2.4.2. Droplet size

Droplet size of the emulsions was measured by laser diffraction in a Mastersizer 2000 (Malvern Instruments, Ltd., Worcestershire, UK) on days 0 and 12 using the method described by Let, Jacobsen, Meyer and Horn et al. [26,27] Results were presented as the surface weighted (D[3,2]) and volume weighted (D[4,3]) mean diameter, which were calculated according to the equation 2 and 3, respectively:

$$D[3,2] = \Sigma nidi^3 / \Sigma nidi^2 \quad \text{(Equation 2)}$$

$$D[4,3] = \Sigma nidi^4 / \Sigma nidi^3 \quad \text{(Equation 3)}$$

where n is the number of droplet with a specific diameter and d is the diameter. Measurements were carried out as duplicates.

2.4.3. Apparent viscosity

Apparent viscosity was measured using a stress-controlled rheometer (Stresstech, Reologica Instruments AB, Lund, Sweden) on days 1 and 12. Rheometer was equipped with a CC25 standard bob cup system in a temperature vessel. 15 ml of emulsion were measured over a shear stress range from 0.0125 to 200 Pa at 25°C. Results were calculated on a specific shear rate (20 s^{-1}) for each emulsion in Pascal second (Pa·s). Samples were measured in duplicates.

2.4.4. Zeta potential

Zeta potential measurements were done using Zetasizer Nano 2S (Malvern Instruments, Ltd.) in order to determine the surface charge of the emulsion droplets. Each sample (0.32 g

187 sample) was diluted in distilled water (40 g of distilled water) before measuring and placed in
188 DTS-1070 disposable folded capillary cell (Malvern Instruments, Ltd., United Kingdom). The
189 zeta potential range was set to -100 to +50 mV and measurements were carried out in
190 duplicate at 25°C on day 2.

191 **2.4.5. Relative protein content in the aqueous phase**

192 Protein content of the aqueous phase was measured based on the method described by
193 Jacobsen, Meyer, & Adler-Nissen.[28] In order to separate the aqueous phase, emulsion
194 sample (~20 g) was centrifuged for 10 min at 25,400g and 10 °C (Sorvall RC-6 PLUS,
195 Thermo Fisher Scientific, Osterode, Germany; rotor SS-34). Supernatant (oil phase) was
196 removed by the use of a pipette. The rest was mixed with distilled water (1:2) and then
197 subjected to ultracentrifugation (Beckman Ultracentrifuge L8-60M, Fullerton, CA; rotor 21102)
198 for 16 h at 106,979g and 15 °C. Dumas method (Elementar, Mt. Laurel, NJ, USA) was used
199 for the determination of protein concentration. Approximately 1 g of aqueous phase was
200 placed in the sample tray and further steps were automated including sample combustion in a
201 chamber at a high temperature (900°C) in the presence of oxygen. Content of crude protein
202 was estimated by using a conversion factor (6.25). Protein concentration is reported as
203 percent of aqueous phase. Measurements were carried out in duplicates.

204 **2.5. Methods for lipid oxidation measurements of emulsions**

205 **2.5.1. Primary oxidation products—peroxide value (PV)**

206 Determination of primary oxidation products was done according to the Bligh and Dyer
207 method[29] with slight changes. Lipid extract was prepared using 5 g emulsion for each

208 extraction and a reduced amount of solvent (30.0 mL of methanol and chloroform, 1:1). PV
209 was subsequently measured on the lipid extracts by colorimetric determination of iron
210 thiocyanate on a spectrophotometer (Shimadzu, UV mini 1240, Kyoto, Japan) at 500 nm, as
211 described by Shantha and Decker.[30] Measurements were carried out in duplicate.

212 **2.5.2. Tocopherol content - HPLC**

213 Tocopherol content of emulsions was determined by HPLC (Agilent 1100 Series; Column:
214 Waters Spherisorb 3 µm Silica; 4.6×150 mm). Tocopherol analysis was carried out according
215 to the official AOCS method[31] using lipid extracts (see Section 2.5.1) that were further
216 evaporated and re-dissolved in heptane. Measurements were carried out in duplicates.

217 **2.5.3. Secondary oxidation products—Dynamic Head Space GC-MS**

218 Volatile secondary oxidation products were analyzed according to the method described by
219 Yesiltas et al.[32] Emulsion samples (approximately 4 g) were mixed with 2 mL antifoam and 10
220 mL distilled water in a 100 mL purge bottle. The bottle was heated in a water bath at 60 °C
221 while purging with nitrogen (flow 150 mL/min, 30 min). Volatile compounds were trapped on
222 Tenax GR tubes. The volatiles were separated in a gas chromatograph (Agilent
223 Technologies, 6890N Network GC System, DE, USA) on a 30 m DB 1701 fused silica
224 capillary column (0.25 mm i.d., 1 µm film thickness; Agilent Technologies, J&W GC Columns,
225 CA, USA). The oven program had an initial temperature of 45 °C for 5 min, increasing with 1.5
226 °C/min until 55 °C, with 2.5 °C/min until 90 °C, and with 12.0 °C/min until 220 °C, where the
227 temperature was held for 4 min. The individual compounds were analyzed by mass-
228 spectrometry (Agilent 5973 Network Mass Selective Detector, Agilent Technologies, 70 eV;

mass to charge ratio scan between 30 and 250) and identified by MS-library searches (Wiley 138 K, John Wiley and Sons, Hewlett-Packard). The volatile compounds 2-ethyl-furan, 1-penten-3-one, 1-penten-3-ol, *(E)*-2-pentenal, hexanal, *(E)*-2-hexenal, *(Z)*-4-heptenal, 2-pentyl-furan, *(E)*-2-heptenal, benzaldehyde, *(E,E)*-2,4-heptadienal, nonanal and *(E,Z)*-2,6-nonadienal were selected for quantification and analyzed in all emulsions. In the optimization study, calibration curve was prepared by injecting standards directly on the TENAX GR tubes. Another calibration curve was prepared for the samples in the second part of this study by injecting standards into an emulsion produced according to the optimal formula (CAS and commercial DATEM were used as emulsifier). Then volatiles were collected the same way as the emulsion samples. This was carried out in order to maintain similar release conditions for standard volatile compounds and volatiles in the emulsion samples.

2.6. Response surface methodology and statistical analysis

Statgraphics XVII (Statpoint Technologies, Inc., Virginia, USA) was used to generate the statistical analysis and the regression models for the parameters, which were used for determining physical and oxidative stability of the emulsions produced for the recipe optimization study. Firstly, the output variables (Y: viscosity at day 1, droplet size at day 1, creaming at day 12, peroxide values at day 12 and volatile compounds at day 12) were related to the input variables (X: fish oil content, total emulsifier content and ratio between CAS and DATEM) by second-order polynomial equation as follows, equation 4:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i<j}^{3} b_{ij} X_i X_j \quad \text{(Equation 4)}$$

where the coefficients bi and bii are related to the linear and quadratic effects, respectively, of each input factor on the output variable and the cross-product coefficients bij represent the interactions between two input variables. Secondly, the analysis of variance (ANOVA) was carried out. The significance of all terms in the models was evaluated statistically at the confidence level 1-α = 95%. Contour maps were drawn using the regression coefficients. Optimal values for each input variables, which maximized the quality of the emulsions in terms of physical and oxidative stability, were determined for each output variable by using RSM.[33]

2.7. Principle component analysis (PCA)

Principal component analysis (PCA) was done by Latentix 2.12 (LatentiX, Copenhagen, Denmark) for the emulsions produced for the recipe optimization study. The PCA was carried out with the emulsions as objects and creaming, viscosity, droplet size, zeta potential, peroxide value, alpha-tocopherol and volatile compounds as variables. Data were autoscaled to make the variables contribute equally to the model.

3. Results and Discussion

3.1. Selection of optimal formula

An optimal formula was selected according to the results of physical and oxidative parameters of the emulsions, which were produced based on the Box-Behnken`s experimental design. Results were analyzed using RSM as described in detail by Yesiltas et al.,[34] and optimal formula was determined as follows: 70% fish oil, 2.8% total emulsifier and 2 as the ratio of CAS to commercial DATEM. As this study was performed as a preliminary study for finding

the optimal recipe, detailed results are shown in the supplementary material. Overall, emulsion viscosity was significantly increased when increasing the amount of fish oil. Creaming and zeta potential of the droplets significantly decreased with increased fish oil content and total emulsifier content. Droplet size significantly decreased with increasing fish oil content, total emulsifier content and the ratio between CAS and DATEM. Additionally, the ratio between CAS and DATEM played a significant role for oxidative stability; having higher amount of CAS resulted in less formation of primary and secondary oxidation products (see the supplementary material).

3.2. Physical characterization of emulsions produced with modified DATEMs

Physical characteristics of the emulsions were identified according to the droplet size, apparent viscosity, zeta potential, and relative protein content in the aqueous phase (Table 2). There was no creaming for these emulsions during 12 days of storage.

3.2.1. Droplet size

During 12 days of storage, surface weighed (D[3,2]) mean diameters did not change significantly except for DATEM_C14_60% (from 2.46±0.01 to 2.49±0.01 μm) as well as volume weighed (D[4,3]) mean diameters for DATEM_C14_60% (from 2.87±0.01 to 2.89±0.00 μm) and DATEM_C12_30% (from 2.81±0.02 to 2.75±0.00 μm) (Table 2). Increased amount of both modified DATEMs (DATEM_C12 and DATEM_C14) led to significantly larger droplet sizes (both in D[3,2] and D[4,3] mean diameters) compared to commercial DATEM emulsion at day 1. This was also the case for the emulsions produced with commercial DATEM and caffeic acid. Compared to commercial DATEM emulsion, only

DATEM_C12_10% and DATEM_C14_10% had similar and significantly smaller droplets, respectively. These differences could be due to the different chain lengths of DATEM, which can affect the adsorption behavior of the emulsifiers by increasing the lipophilic characteristic of the emulsifiers. On the other hand, when caffeic acid is attached to DATEM, it increases the size of the head group of the DATEM emulsifier, which is then expected to affect the HLB of the emulsifier.[35] This could lead to higher hydrophilic characteristic; thereby, increase the emulsifier`s affinity towards water phase, which slows down the adsorption of modified DATEMs at the oil-water interface compared to DATEM. Other studies also reported that phenolic compounds interacted with emulsifiers (e.g. proteins) in emulsions and this might have affected the functionality as well as emulsifying properties of emulsifiers at the oil-water interface.[15,36,37] Mattia et al. observed that catechin addition into emulsions emulsified with Tween20/β-lactoglobulin resulted in larger droplet sizes compared to control, which indicated a possible negative effect of phenols on emulsifying activities of emulsifiers.[38] This phenomenon was attributed to having protein-protein interactions mediated by catechin in 20% olive oil-in-water emulsions. In our study, increase in droplet size could be also explained by protein-DATEM/modified DATEM interactions, which might have been favored in the presence of caffeic acid and carboxylic acid units in the structures of DATEM.[39] It was shown that DATEM has a strong ability towards the formation of hydrogen bonds with amidic groups of the gluten-proteins.[35]

Thus, the smaller droplet size for the emulsions with lower amounts of modified DATEMs (DATEM_C12_10% and DATEM_C14_10%) compared to higher amounts of modified DATEMs (DATEM_C12_60% and DATEM_C14_60%) could be explained by the higher

amount of commercial DATEM which exhibited higher surface activity than DATEM C12 and C14. However, this cannot explain the differences observed in droplet size for DATEM_C14_10% emulsion and the DATEM emulsion.

3.2.2. Apparent viscosity

All the emulsions were non-Newtonian and showed shear thinning behavior. Same characteristics were also reported by Yesiltas et al. for high fat oil-in-water emulsions stabilized with CAS and alginates.[32] Apparent viscosity of the samples showed that 60% replacement of the DATEM with modified DATEMs provided significantly less viscous emulsions. This was the case also for the emulsions which had 10% of its DATEM replaced by DATEM C14. Emulsions produced with commercial DATEM and caffeic acid also had significantly lower viscosities compared to DATEM emulsion without caffeic acid. This shows that the decrease in viscosity was affected by the presence of caffeic acid in the emulsions, which could be related to the changes in droplet size discussed under the section 3.2.1. Changes in the viscosity correlated well with the droplet size results, where viscosity decreased with the larger droplet size (except for emulsion DATEM_C14_10%). This could be explained by the fact that smaller droplet sizes led to a close packing of emulsion droplets, and thereby larger interfacial area, which can be related to increased friction forces between droplets at an expanded surface-to-volume ratio of the dispersed phase. This could result in less mobility of the oil droplets in the emulsion and therefore higher viscosity.[32,40]

3.2.3. Zeta potential

Zeta potential results are a measure of the surface charge of the droplets in the emulsions. Surface charge of the emulsions was negative due to molecular charges of both CAS[41] and DATEM[42] at pH 7. Zeta potential values for emulsions ranged between -60.6 ± 1.1 and -75.3 ± 2.3 mV (Table 2). Only DATEM_C12_60% and DATEM_caf_high were found to be significantly different compared to DATEM emulsion. Moreover, increased amount of modified DATEM resulted in more negatively charged oil droplets. This could be due to having higher concentration of caffeic acid at the interface which interacted with iron as well and became more negatively charged. This was also observed in another study where addition of iron caused more negatively charged droplets in citrem-stabilized emulsions (pH 6) where phenolic compounds were also present. It was reported that emulsions with added caffeic acid had much higher negative charge compared to emulsions with added rutin or naringenin, after iron was added.[15] In addition, the anionic nature of modified DATEM could have contributed to the increased negative charge of oil droplets.

3.2.4. Relative protein content in the aqueous phase

Results of relative protein content in the water phases of the emulsions are presented in Table 2. The protein content in the water phase is a measure of the amount of protein (CAS), which has been replaced by modified DATEM at the interface or nonadsorbed proteins in the aqueous phase. It was observed that emulsions produced with DATEM C14 replaced more CAS compared to DATEM C12. This could be due to the effect of chain length on surface activity of modified DATEMs; meaning DATEM C14 had higher affinity to oil-water interface compared to DATEM C12 in 70% fish oil-in-water emulsions. This was the case for both 10% and 60% replacement of DATEM with modified DATEMs. Protein content in the aqueous

phase was similar for DATEM_C14_60% and DATEM, which confirmed that the replacing 60% of the DATEM with DATEM C14 did not affect the protein replacement at the oil-water interface. On the other hand, DATEM_C12_10% had less protein in the aqueous phase, thereby more proteins at the oil-water interface compared to DATEM_C14_10%. This could be attributed to lower surface activity of DATEM C12 compared to DATEM C14 or DATEM.

On the other hand, results from emulsions with commercial DATEM and added free caffeic acid showed that the amount of CAS in the water phase was lower compared to the control (emulsion with commercial DATEM and no caffeic acid), which suggested that the caffeic acid presence decreased the adsorption of commercial DATEM at the oil-water interface. Even though DATEM_caf_low and DATEM_caf_high had larger droplet size and thereby lower surface area compared to emulsion with only DATEM, these emulsions had more CAS adsorbed at the interface. This decreased amount of CAS replacement by commercial DATEM could, presumably, be explained by the interactions between caffeic acid and commercial DATEM, which resulted in lower amounts of CAS replacement at the oil water interface compared to control. Sørensen et al. reported that partitioning results showed that caffeic acid and ester of caffeic acid (caffeates) had different distribution in the aqueous phase of Citrem and Tween 80 stabilized emulsions.[43] Less caffeic acid and caffeates were present in the aqueous phase of Tween 80 stabilized emulsions, which indicated that Tween 80 had a stronger interaction with caffeic acid and caffeates compared to Citrem. Thus, it is possible that free caffeic acid had interactions with DATEM, which affected its surface activity.

3.2.5. Colour

Iron-caffeic acid complex was formed after iron was added into emulsions produced with modified DATEM which had caffeic acid in their structure (Figure 1) as well as emulsions produced with commercial DATEM and free caffeic acid. It could easily be observed as a color change to light gray. It is known that phenols interact with iron and form phenol-iron complexes due to their metal chelating properties.[15,44]

3.3. Lipid oxidation in emulsions produced with modified DATEMs

Oxidative stability of emulsions was evaluated by measuring the formation of primary and secondary oxidation products during 12 days of storage as well as the consumption of tocopherols.

3.3.1. Primary oxidation products

Peroxide value of the emulsions had a significant increase during 12 days of storage (Fig. 2). The most oxidized emulsion was DATEM without caffeic acid with 4.7±0.1 peroxides/ kg oil at day 12. High fat (70%) fish oil-in-water emulsions produced with CAS and succinylated alginate or only CAS showed better oxidative stability (1.7±0.2 and 1.8±0.2 peroxides/ kg oil, respectively) compared to emulsions produced with CAS and DATEM, whereas emulsions produced with CAS and dodecyl succinylated alginate or CAS and commercial alginate (8.0±0.8 and 9.0±1.4 peroxides/ kg oil, respectively) showed less oxidative stability.[32] Emulsions with the same oil content and produced with CAS and phosphatidylcholine had values ranging between 3.5–5.0 which was similar to emulsions with CAS and DATEM.[32,34] These differences could be due to antioxidative activity of different emulsifiers as well as different amounts of total emulsifier and the ratios between emulsifiers.

Emulsions were oxidatively more stable when the concentration of caffeic acid was increased for all emulsifier combinations no matter whether DATEM was modified or not. Emulsions with the highest concentration of modified DATEMs (DATEM_C12_60% and DATEM_C14_60%) were more oxidatively stable (0.8±0.0 and 0.8±0.0 peroxides/ kg oil) compared to the DATEM_caf_high (1.1±0.0 peroxides/ kg oil) which had the same concentration of caffeic acid added in the free form at day 12. Conversely, this effect was observed vice versa at the lower concentration of caffeic acid. DATEM_C12_10% and DATEM_C14_10%, which had the caffeic acid attached to the emulsifier itself, oxidized significantly more than DATEM_caf_low, which had the same concentration of caffeic acid added in the free form. This led to the conclusion that having caffeic acid attached to the emulsifier, and when using a high concentration of the modified emulsifier, restrained the formation of peroxides compared to the emulsions produced with the commercial DATEM and equivalent concentrations of caffeic acid in free form.

3.3.2. Tocopherol content

Alpha-, gamma- and delta-tocopherols were quantified on day 0 to be in the range of 145.1 ± 8.0 – 161.0 ± 3.5, 67.8 ± 2.1 – 73.0 ± 0.2 and 27.5 ± 0.6 – 29.7 ± 0.2 μg/g emulsion, respectively (data not shown). Beta-tocopherol content was very low (1-2 μg/g emulsion); therefore, it was not considered. Alpha-tocopherol content of the emulsion samples significantly decreased at day 12 compared to day 0 except for DATEM_C12_10%. Likewise, delta-tocopherol content of the emulsions was also decreased from day 0 to day 12 except for DATEM_C12_10% and DATEM_C12_60%. However, gamma-tocopherol content was not significantly different when days 0 and 12 were compared. Tocopherols are natural chain-

419 breaking antioxidants, which react with free radicals by donating hydrogen atoms. Therefore,
420 consumption of tocopherols indicates that some of the free radicals were deactivated by
421 tocopherols, which presumably helped to decrease the formation of oxidation products.
422 Emulsions which had decrease in the tocopherol content would have had higher levels of
423 oxidation products formed during the storage. Moreover, in the presence of caffeic acid,
424 tocopherols can be regenerated through hydrogen donation mechanism. Thus, it was
425 reported that the consumption rate of tocopherols was faster in the control sample compared
426 to caffeic acid supplemented fish muscle, which confirmed that caffeic acid efficiently
427 protected tocopherols for retarding the depletion of alpha-tocopherol by hydrogen
428 donation.[45,46]

429 **3.3.3. Volatile secondary oxidation products**

430 Emulsions showed a similar trend in the content of most of the volatile compounds during
431 storage. Therefore, sum of the volatile compounds formed during storage was shown in Fig.
432 3a, together with most abundant two volatile compounds, (*E,E*)-2,4-heptadienal and 1-penten-
433 3-ol in 3b,c (see supplementary material for some of the other individual volatile compounds).
434 Although there were some differences, in general terms similar trends were observed for 1-
435 penten-3-ol, (*E,E*)-2,4-heptadienal and the sum of volatiles. Emulsions produced with
436 modified DATEMs showed better oxidative stability compared to the control (commercial
437 DATEM without added caffeic acid). Moreover, added caffeic acid in low concentration into
438 the emulsion showed pro-oxidative effect (see DATEM_caf_low). Prooxidant effect of caffeic
439 acid was also reported in 10% fish oil-in-water emulsions produced with Tween 80 with added
440 iron at pH 6.[15] It was attributed to caffeic acid`s ability to reduce endogenous Fe^{3+} in the fish

441 oil or emulsifier to Fe^{2+}.[15,23] Emulsions produced with the modified DATEMs showed better
442 oxidative stability compared to emulsions produced with commercial DATEM with added
443 equivalent concentration of caffeic acid both at low and high concentrations of caffeic acid.
444 This suggests that having caffeic acid attached to the emulsifier improved oxidative stability
445 compared to the emulsions containing free caffeic acid in the same concentration, which
446 presumably support the claim that oxidation is initiated at the interface. Having caffeic acid
447 located at the interface improved oxidative stability of the emulsions due to their radical
448 trapping mechanisms. As shown in Fig. 3, DATEM_C14 showed slightly better oxidative
449 stability compared to DATEM_C12, which could be due to the effect of the chain length on
450 their affinity to the oil-water interface. Protein content in the aqueous phase (see section
451 3.2.4) supported that DATEM_C14 replaced more CAS at the interface when compared to
452 DATEM_C12. This could be the reason for the higher oxidative stability of DATEM_C14
453 emulsion.

454 As a conclusion, the tandem use of CAS, commercial and modified DATEMs allowed
455 production of physically and oxidatively stable 70% fish oil-in-water emulsions. DATEM
456 modified with caffeic acid and C14-alkyl chain replaced more CAS from the oil-water interface
457 compared to DATEM modified with caffeic acid and C12-alkyl chain in 70% fish oil-in-water
458 emulsions. Covalent attachment of caffeic acid to DATEM significantly improved oxidative
459 stability compared to the emulsions produced with physical combinations of commercial
460 DATEM and caffeic acid. These results highlight the importance of bringing antioxidants to the
461 oil-water interface by the new approach of attaching antioxidants to an emulsifier molecule.
462 When 60% of the commercial DATEM was substituted with either of the modified DATEMs

(C12 or C14), viscosity was significantly decreased compared to emulsions with commercial DATEM, which might make high fat emulsions` incorporation into low viscosity food systems easier.

Abbreviations Used

CAS – sodium caseinate

DATEM – diacetyl tartaric acid esters of mono- and diglycerides

DHA – docosohexaenoic acid

DHS – dynamic head space

EPA – eicosapentaenoic acid

HPLC – high pressure liquid chromatography

LC PUFAs – long chain poly unsaturated fatty acids

LMWEs – low molecular weight emulsifiers

PV – peroxide value

PCA – principle component analysis

RSM – response surface methodology

Acknowledgments

We would like to thank Arla Foods Ingredients amba and Dupont for donating sodium caseinate and DATEM, respectively. We would also like to thank Maritex A/S, Norway for providing cod liver fish oil. Furthermore, we are grateful to Inge Holmberg, Lis Berner, Yuka Omura Lund and Thi Thu Trang Vu for their skillful work in the laboratory. Finally, we would like to thank the Danish Council for Independent Research Technology and Production Sciences for financing the project Mapping and Characterizing of Lipid Oxidation in Emulsified Systems (MAPOX).

The authors have declared no conflict of interest.

Supporting Information description

Table 1. Box-behnken`s design for emulsions

Figure 1. Principle component analysis of the emulsions for the optimization study

Figure 2. Response surface methodology results for the optimization study: a) Pareto charts, b) contour plots

Figure 3. Volatile compounds formed during storage of emulsions produced with modified DATEMs: a) 1-penten-3-one, b) 2-pentenal, and c) hexanal

References

(1) Calder, P. C.; Yaqoob, P. Marine omega-3 fatty acids and coronary heart disease. *Curr. Opin. Cardiol.* **2012**, *27*, 412-419.

(2) Song, C.; Shieh, C.; Wu, Y.; Kalueff, A.; Gaikwad, S.; Su, K. The role of omega-3 polyunsaturated fatty acids eicosapentaenoic and docosahexaenoic acids in the

499 treatment of major depression and Alzheimer's disease: Acting separately or
500 synergistically? *Prog. Lipid Res.* **2016**, *62*, 41-54.
501 (3) EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific opinion on
502 dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty
503 acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA J.* **2010**, *8*,
504 1461-1568.
505 (4) Cheatham, C. L.; Colombo, J.; Carlson, S. E. n-3 Fatty acids and cognitive and visual
506 acuity development: methodologic and conceptual considerations. *Am. J. Clin. Nutr.*
507 **2006**, *83*, 1458S-1466S.
508 (5) Wysoczański, T.; Sokoła-Wysoczańska, E.; Pékala, J.; Lochyński, S.; Czyz, K.;
509 Bodkowski, R.; Librowski, T. Omega-3 fatty acids and their role in central nervous
510 system – A review. *Curr. Med. Chem.* **2016**, *23–8*, 816–831.
511 (6) Nichols, P. D.; McManus, A.; Krail, K.; Sinclair, A. J.; Miller, M. Recent advances in
512 omega-3: Health benefits, sources, products and bioavailability. *Nutrients.* **2014**, *6*,
513 3727-3733.
514 (7) Jacobsen, C.; Let, M. B.; Nielsen, N. S.; Meyer, A. S. Antioxidant strategies for
515 preventing oxidative flavour deterioration of foods enriched with n-3 polyunsaturated
516 lipids: a comparative evaluation. *Trends Food Sci. Technol.* **2008**, *19*, 76-93.
517 (8) Berton-Carabin, C. C.; Ropers, M. H.; Genot, C. Lipid Oxidation in Oil-in-Water
518 Emulsions: Involvement of the Interfacial Layer. *Compr. Rev. Food Sci. Food Saf.*
519 **2014**, *13 (5),* 945-977.
520 (9) Jacobsen, C. Some strategies for the stabilization of long chain n-3 PUFA-enriched
521 foods: A review. *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 1853-1866.

(10) Genot, C.; Meynier, A.; Riaublanc, A. Lipid oxidation in emulsions. In *Lipid oxidation pathways;* Kamal-Eldin, A.; AOCS Press: Champaign, IL, 2003; pp. 190-244).

(11) García-Moreno, P. J.; Horn, A. F.; Jacobsen, C. Influence of casein-phospholipid combinations as emulsifier on the physical and oxidative stability of fish oil-in-water emulsions. *J. Agric. Food Chem.* **2014**, *62*, 1142-1152.

(12) McClements, D. J.; Bai, L.; Chung, C. Recent Advances in the Utilization of Natural Emulsifiers to Form and Stabilize Emulsions. *Annu. Rev. Food Sci. Technol*. **2017**, *8*, 205-236.

(13) Fang, Y.; Dalgleish, D. G. Casein adsorption on the surfaces of oil-in-water emulsions modified by lecithin. *Colloids Surf. B Biointerfaces.* **1993**, 357-364.

(14) Munk, M. B.; Larsen, F. H.; van den Berg, F. W. J.; Knudsen, J. C.; Andersen M. L. Competitive Displacement of Sodium Caseinate by Low-Molecular-Weight Emulsifiers and the Effects on Emulsion Texture and Rheology. *Langmuir.* **2014**, *30*, 8687-8696.

(15) Sørensen, A.-D. M.; Haahr, A.-M.; Becker, E. M.; Skibsted, L. H.; Bergenståhl, B.; Nilsson, L.; Jacobsen, C. Interactions between Iron, Phenolic Compounds, Emulsifiers, and pH in Omega-3-Enriched Oil-in-Water Emulsions. *J. Agric. Food Chem.* **2008,** *56,* 1740-1750.

(16) Laguerre, M.; Giraldo, L. J. L.; Lecomte, J.; Figueroa-Espinoza, M. C.; Barea, B.; Weiss, J.; Decker, E. A.; Villeneuve, P. Chain length affects antioxidant properties of chlorogenate esters in emulsion: The cutoff theory behind the polar paradox. *J. Agric. Food Chem.* **2009**, *57*, 11335-11342.

(17) Laguerre, M.; Giraldo, L. J. L.; Lecomte, J.; Figueroa-Espinoza, M.- C.; Baréa, B.; Weiss, J.; Decker, E. A.; Villeneuve, P. Relationship between hydrophobocity and antioxidant ability of “phenolipids” in emulsion: A parabolic effect of the chain length of rosmarinate esters. *J. Agric. Food Chem.* **2010**, *58*, 2869–2876.

(18) Alemán, M.; Bou, R.; Guardiola, F.; Durand, E.; Villeneuve, P.; Jacobsen, C.; Sørensen, A.-D. M. Antioxidative effect of lipophilized caffeic acid in fish oil enriched mayonnaise and milk. Food *Chem.* **2015**, *167*, 236-244.

(19) Sørensen, A.-D. M.; Durand, E.; Laguerre, M.; Bayrasy, C.; Lecomte, J.; Villeneuve, P.; Jacobsen, C. Antioxidant properties and efficacies of synthesized alkyl caffeates, ferulates and coumarates. *J. Agric. Food Chem*. **2014**, *62*, 12553−12562.

(20) Sørensen, A.-D. M.; Lyneborg, K. S.; Villeneuve, P.; Jacobsen, C. Alkyl chain length impacts the antioxidative effect of lipophilized ferulic acid in fish oil enriched milk. *J. Funct. Foods.* **2015**, *18*, 959–967.

(21) Costa, M.; Losada-Barreiro, S.; Paiva-Martins, F.; Bravo-Díaz, C.; Romsted L. S. A direct correlation between the antioxidant efficiencies of caffeic acid and its alkyl esters and their concentrations in the interfacial region of olive oil emulsions. The pseudophase model interpretation of the ‘cut-off’ effect. *Food Chem.* **2015***, 175*, 233-242.

(22) Costa, M.; Losada-Barreiro, S.; Paiva-Martinsa, F.; Bravo-Díaz, C. Physical evidence that the variations in the efficiency of homologous series of antioxidants in emulsions are a result of differences in their distribution. *J. Sci. Food Agric.* **2016**, *97*, 564-571.

(23) Gülcin, I. Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). *Toxicology.* **2006**, *217*, 213-220.

(24) Anankanbil, S.; Pérez, B.; Fernandes, I.; Widzisz, K. M.; Wang, Z.; Mateus, N.; Guo, Z. A new group of synthetic phenoliccontaining amphiphilic molecules for multipurpose applications: Physico-chemical characterization and cell-toxicity study. *Sci. Rep.* **2018**, *8*, 832.

(25) Anankanbil, S.; Pérez, B.; Yang, J.; Banerjee, C.; Guo, Z. A novel array of interface confined molecules: Assembling natural segments for delivery of multi-functionalities. *J. Colloid Interface Sci.* **2017**, *508*, 230-236.

(26) Let, M. B.; Jacobsen, C.; Meyer, A. S. Lipid oxidation in milk, yoghurt, and salad dressing enriched with neat fish oil or pre emulsified fish oil. *J. Agric. Food Chem.* **2007***, 55*, 7802-7809.

(27) Horn, A. F.; Nielsen, N. S.; Andersen, U.; Søgaard, L. H.; Horsewell, A.; Jacobsen, C. Oxidative stability of 70% fish oil-in-water emulsions: Impact of emulsifiers and pH. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 1243-1257.

(28) Jacobsen, C.; Meyer, A. S.; Adler-Nissen, J. Oxidation mechanisms in real food emulsions: Method for separation of mayonnaise by ultracentrifugation. *J. Food Lipids* **1998**, *5*, 87-101.

(29) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911-917.

(30) Shantha, N. C.; Decker, E. A. Rapid, sensitive, iron based spectrophotometric methods for determination of peroxide values of food lipids. *J. AOAC Int.* **1994**, *77*, 421-424.

589 (31) AOCS Official Method Ce 8-89. Determination of tocopherols and tocotrienols in
590 vegetable oils and fats by HPLC. AOCS: Champaign, IL, USA; 1998.
591 (32) Yesiltas, B.; Sørensen, A.-D. M.; García-Moreno, P. J.; Anankanbil, S.; Guo, Z.;
592 Jacobsen, C. Combination of sodium caseinate and succinylated alginate improved
593 stability of high fat fish oil-in-water emulsions. Food Chem. **2018**, *255*, 290-299.
594 (33) Myers, R. H.; Montgomery, D. C.; Anderson-Cook, C. M. Response Surface
595 Methodology, Process and Product Optimization Using Designed Experiments, Third
596 ed.; Hoboken; Wiley: New Jersey, 2002.
597 (34) Yesiltas, B.; Sørensen, A.-D. M.; García-Moreno, P. J.; Akoh, C. C.; Jacobsen,
598 C. Physical and oxidative stability of high fat fish oil-in-water emulsions stabilized with
599 sodium caseinate and phosphatidylcholine as emulsifiers. Submitted to Food Chem.
600 **2018**.
601 (35) Gaupp, R.; Adams, W. Di-acetyltartaric esters of monoglycerides (DATEM) and
602 associated emulsifiers in bread making. In *Emulsifiers in Food Technology*; Whitehurst,
603 R. J.; Blackwell Publishing Ltd.: Northampton, United Kingdom, 2004; pp. 86-109.
604 (36) Pripp, A. H.; Vreeker, R.; van Duynhoven, J. Binding of olive oil phenolics
605 to food proteins. *J. Sci. Food Agric.* **2005***, 85*, 354-362.
606 (37) Mattia, C. D.; Paradiso, V. M.; Andrich, L.; Giarnetti, M.; Caponio, F.; Pittia, P.
607 Effect of Olive Oil Phenolic Compounds and Maltodextrins on the Physical Properties
608 and Oxidative Stability of Olive Oil O/W Emulsions. *Food Biophys.* **2014**, *9*, 396-405.

(38) Mattia C. D. D.; Sacchetti, G.; Mastrocola, D.; Sarker, D. K.; Pittia, P. Surface properties of phenolic compounds and their influence on the dispersion degree and oxidative stability of olive oil O/W emulsions. *Food Hydrocoll.* **2010**, *24*, 652-658.

(39) Köhler, P. Study of the Effect of DATEM. 3: Synthesis and Characterization of DATEM Components. *LWT.* **2001**, *34*, 359-366.

(40) Pal, R. Effect of Droplet Size on the Rheology of Emulsions. *AIChE J.* **1996**, *42(11),* 3181-3190.

(41) Capron, I.; Costeux, S.; Djabourov, M. Water in water emulsions: Phase separation and rheology of biopolymer solutions*. Rheol. Acta.* **2001**, *40*, 441-456.

(42) McClements, D. J. Emulsion Ingredients. In *Food Emulsions: Principles, Practices, and Techniques*, Third ed.; CRC Press: Boca Raton, 2015.

(43) Sørensen, A.-D. M.; Villeneuve, P.; Jacobsen, C. Alkyl caffeates as antioxidants in O/W emulsions: Impact of emulsifier type and endogenous tocopherols. *Eur. J. Lipid Sci. Technol.* **2017**, *119*, 1600276.

(44) Genaro-Mattos, T. C.; Maurício, A. Q.; Rettori, D.; Alonso, A.; Hermes-Lima, M. Antioxidant Activity of Caffeic Acid against Iron-Induced Free Radical Generation—A Chemical Approach. *PLoS ONE* **2015**, *10*(6), e0129963.

(45) Iglesias, J.; Pazos, M.; Andersen, L.; Skibsted, L. H.; Medina, I. Caffeic acid as antioxidant in fish muscle: Mechanism of synergism with endogenous ascorbic acid and a-tocopherol. *J. Agric.Food Chem.* **2009**, *57*(2), 675-681.

(46) Medina, I.; Undeland, I.; Larsson, K.; Storrø, I.; Rustad, T.; Jacobsen, C.; Kristinová, V.; Gallardo, J. M. Activity of caffeic acid in different fish lipid matrices: A review. *Food Chem.* **2012**, *131*, 730-740.

Figure captions

Figure 1. Molecular structures of modified DATEMs (DATEM modified with caffeic acid and C12 or C14 alkyl chain lengths)

Figure 2. Primary oxidation products formed during the storage of the emulsion samples. Sample codes refer to Table 1.

Figure 3. Volatile secondary oxidation products formed during the storage of the emulsion samples a) sum of the volatile compounds, b) (*E,E*)-2,4-heptadienal, and c) 1-penten-3-ol. Sample codes refer to Table 1.

Table 1. Experimental design for determining the effects of having modified DATEMs

Sample codes	Sample descriptions	Commercial DATEM (%)	Modified DATEM (%)	Caffeic acid (%)
DATEM	only DATEM (commercial)	0.93	-	-
DATEM_C12_10%	10% of DATEM was replaced by DATEM C12	0.84	0.09	0.03*
DATEM_C12_30%	30% of DATEM was replaced by DATEM C12	0.65	0.28	0.09*
DATEM_C12_60%	60% of DATEM was replaced by DATEM C12	0.37	0.56	0.18*
DATEM_C14_10%	10% of DATEM was replaced by DATEM C14	0.84	0.09	0.03*
DATEM_C14_60%	60% of DATEM was replaced by DATEM C14	0.37	0.56	0.17*
DATEM_ caf_low	DATEM with added caffeic acid at low conc.	0.93	-	0.03
DATEM_ caf_high	DATEM with added caffeic acid at high conc.	0.93	-	0.17

*Commercial caffeic acid was not added for this sample. Percentages was calculated according to the added modified DATEM, which had caffeic acid attached to the compound itself.

Table 2. Droplet size, viscosity, zeta potential, and protein content in the aqueous phase results of emulsions

	D[3,2] (μm) (Day 1)	D[4,3] (μm) (Day 1)	Apparent viscosity (Pa·s) at 20 s^{-1} (Day 1)	Zeta potential (mV)	Protein in the aqueous phase (%)
DATEM	2.35 ± 0.02^{b}	2.73 ± 0.01^{b}	7.27 ± 0.10^{d}	(-) 63.4 ± 1.3^{cd}	4.85 ± 0.01^{c}
DATEM_C12_10%	2.32 ± 0.02^{b}	2.73 ± 0.01^{b}	7.40 ± 0.24^{de}	(-) 63.6 ± 1.1^{cd}	4.49 ± 0.02^{a}
DATEM_C12_30%	2.43 ± 0.02^{d}	2.81 ± 0.02^{c*}	7.74 ± 0.11^{e*}	(-) 64.4 ± 1.4^{c}	4.70 ± 0.08^{b}
DATEM_C12_60%	2.71 ± 0.00^{f}	3.20 ± 0.00^{f}	5.84 ± 0.06^{b*}	(-) 75.3 ± 2.3^{a}	4.65 ± 0.04^{b}
DATEM_C14_10%	2.20 ± 0.03^{a}	2.57 ± 0.02^{a}	6.49 ± 0.10^{c*}	(-) 60.6 ± 1.1^{d}	5.05 ± -$^{¥}$
DATEM_C14_60%	2.46 ± 0.01^{d*}	2.87 ± 0.01^{d*}	5.89 ± 0.20^{b}	(-) 66.3 ± 2.2^{bc}	4.86 ± 0.07^{c}
DATEM_caf_low	2.39 ± 0.02^{c}	2.78 ± 0.01^{c}	6.84 ± 0.46^{c*}	(-) 64.8 ± 1.7^{c}	4.63 ± 0.04^{b}
DATEM_caf_high	2.63 ± 0.02^{e}	3.14 ± 0.03^{e}	5.37 ± 0.33^{a*}	(-) 68.6 ± 2.4^{b}	4.62 ± 0.11^{b}

*Samples changed significantly during 12 days of storage time.

$^{a\text{-}f}$Letters indicate the significant differences between samples for the same physical parameter.

$^{¥}$This sample has only one replicate, due to a measurement error.

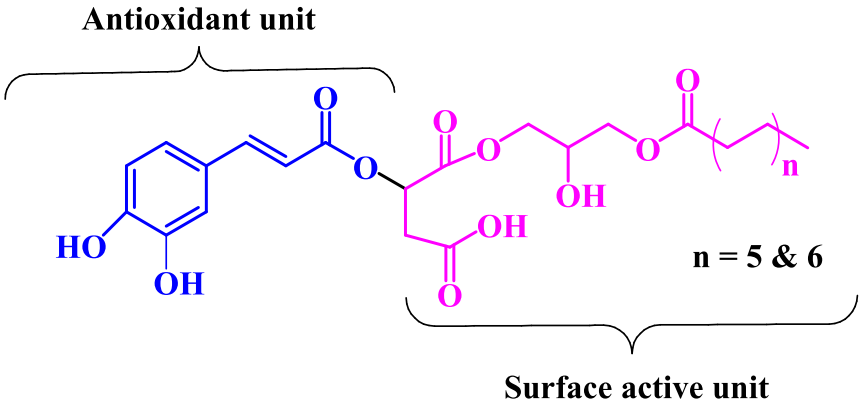

Antioxidant unit
HO
OH
O
O
O
O
OH
O
O
OH
O
n
n = 5 & 6
Surface active unit

Figure 1.

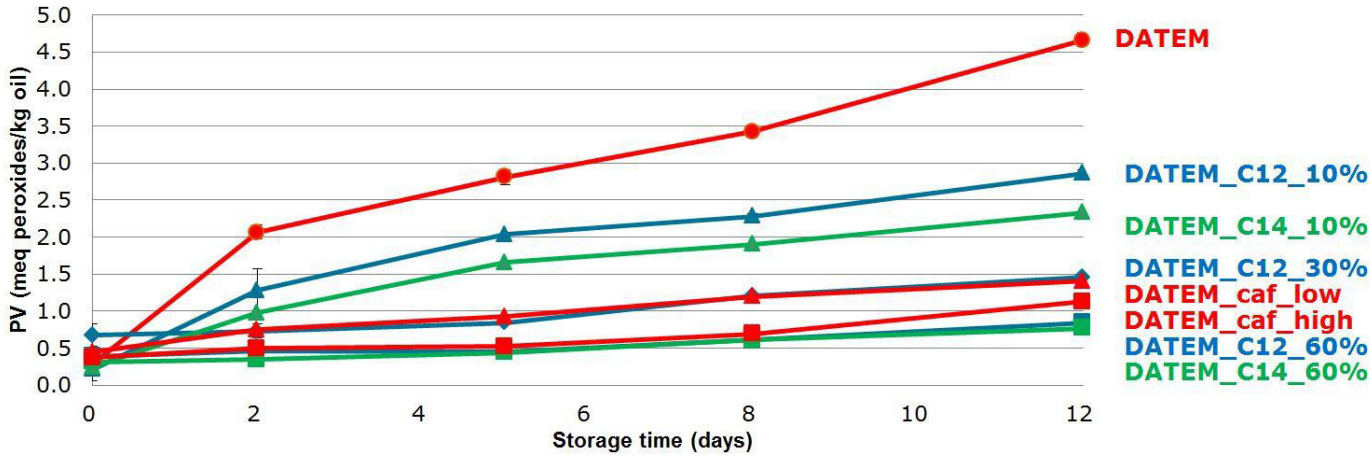
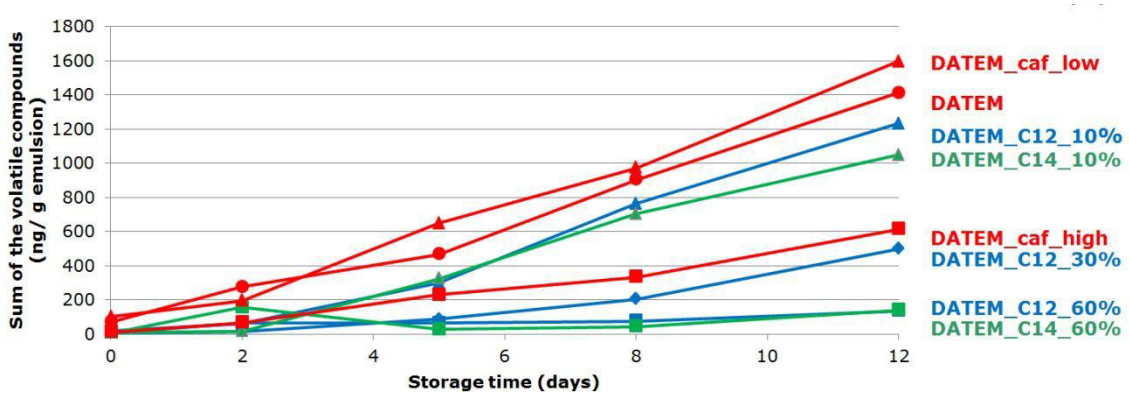
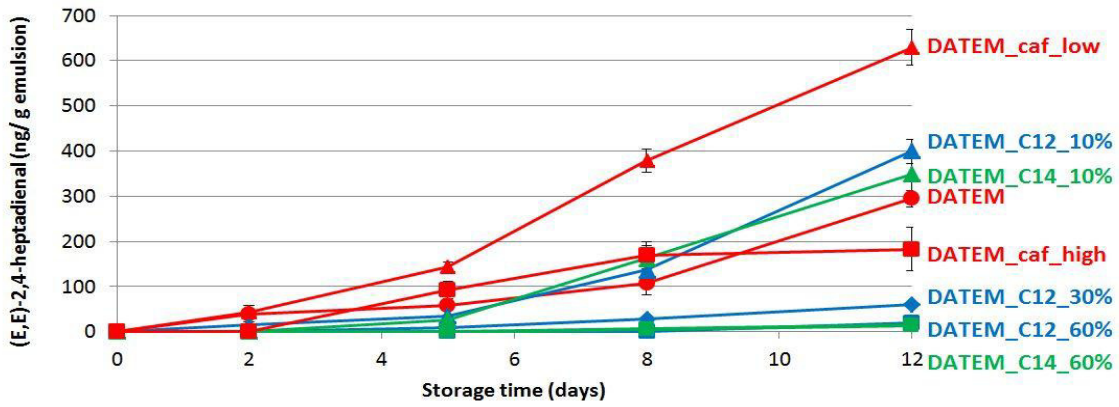

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
PV (meq peroxides/kg oil)
0
2
4
6
8
10
12
Storage time (days)
DATEM
DATEM_C12_10%
DATEM_C14_10%
DATEM_C12_30%
DATEM_caf_low
DATEM_caf_high
DATEM_C12_60%
DATEM_C14_60%

Figure 2.

a)


Sum of the volatile compounds (ng/ g emulsion)
1800
1600
1400
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
Storage time (days)
DATEM_caf_low
DATEM
DATEM_C12_10%
DATEM_C14_10%
DATEM_caf_high
DATEM_C12_30%
DATEM_C12_60%
DATEM_C14_60%

b)


(E,E)-2,4-heptadienal (ng/ g emulsion)
700
600
500
400
300
200
100
0
0
2
4
6
8
10
12
Storage time (days)
DATEM_caf_low
DATEM_C12_10%
DATEM_C14_10%
DATEM
DATEM_caf_high
DATEM_C12_30%
DATEM_C12_60%
DATEM_C14_60%

c)

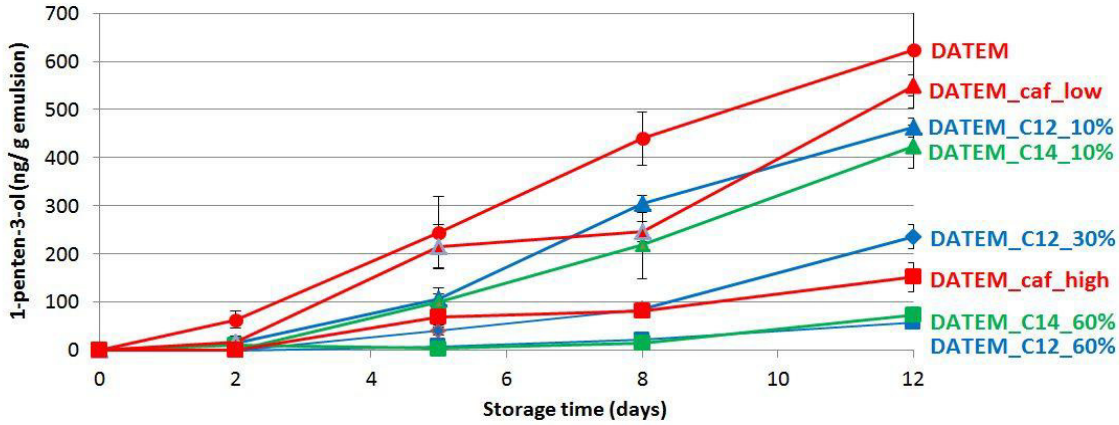

1-penten-3-ol (ng/ g emulsion)
700
600
500
400
300
200
100
0
0
2
4
6
8
10
12
Storage time (days)
DATEM
DATEM_caf_low
DATEM_C12_10%
DATEM_C14_10%
DATEM_C12_30%
DATEM_caf_high
DATEM_C14_60%
DATEM_C12_60%

Figure 3.